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*POSTZYGOTIC ELIMINATION OF GENETIC FACTORS IN ESCHERICHIA COLI**

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Segmental Elimination.—Genetic recombination analysis has been related to a typical haplobiontic life-cycle in *Escherichia coli*, strain K-12: the vegetative proliferation of the haploid phase alternates with occasional syngamy to form a transient diplophase. This, in turn, segregates almost immediately to restore the vegetative haplophase,[1, 2] barring rare diploid exceptions.[3] The exceptional diploids have been disqualified as representative of the primary zygote on two counts: some were homozygous for certain factors, usually heterozygous, and all were hemizygous for the factors *Mal* and *S*. The first peculiarity can be explained if the diploids as recovered are not simply unreduced zygotes but nondisjunctions of segregant genomes after meiosis. A similar process, accelerated by ultraviolet light, also leads to automictic homozygotes from established heterozygotes.[4, 5] The second aberration is probably a feature of all crosses in *E. coli*, strain K-12, and is reflected in anomalies of segregation and mapping.[3–6]

Hemizygosity for the Mal_1 locus was initially inferred from the finding that diploids from crosses of $Mal_1^- \times Mal_1^+$ were invariably pure for this marker, though segregating for many others. The types carrying the Mal^- allele were tested further by a reversion analysis[3, 5] which showed that purity for Mal^- represented a hemizygous, not a homozygous, state at this locus. That is, the diploids are imperfect, and a segment (or chromosome) including the Mal_1 locus is represented only once, though most of the other genetic factors are of course represented twice. The problem is thus narrowed to the contingencies by which the full genetic content of both parents fails to be represented in each diploid. Two hypotheses had been considered: (1) preliminary exclusion of the segment from a gamete or (2) its subsequent elimination from a complete zygote previously formed from intact gametes. The former interpretation, although superficially simpler, was doubted from the first because the diploids were invariably hemizygous for *Mal* but might carry the allele from either one of the two parents. This indicated that, in any

cross, gametes carrying either *Mal* allele were present. No restrictions on compatibility were then known that might account for the failure of zygotes to form from two intact gametes. On the other hand, the postzygotic elimination hypothesis did not explain the unequal proportions of the two hemizygous types. Another factor (*S*) has since been found to be closely linked to *Mal* and to share its behavior in diploids,[5, 6] and a sexual compatibility system has been discovered.[7] The present paper reports evidence on the relationship of elimination of the *Mal-S* segment to the compatability system. In previous reports,[5] diploids have been recorded which were hemizygous for a *Mal* allele from one parent, an *S* allele from the other. These amphitypic diploids argued strongly for the attribution of aberrant hemizygosity to a postzygotic process, a conclusion also fortified by the data of the present paper.

Compatibility.[7, 8]—Sexual interactions in *E. coli* K-12 are now known to be controlled in part by the compatibility status of the parents, which is, in turn, under joint environmental and genetic control. Different sublines of strain K-12 are designated as either F^+ or F^-, as determined by the total sterility of $F^- \times F^-$ crosses, other combinations being compatible. The *F* status is regularly inherited within a clone, but, remarkably, it is contagious between clones, F^- cells becoming rapidly and permanently converted by mixed culture with F^+. However, the infective agent inferred from this epidemiological argument has not been separated from the cells despite assiduous efforts. In the absence of experimental support for the action of a "virus," the contagion of *F* status must be ascribed to an unknown mechanism involving superficial contact of the different cell types.

The compatibility status of the parents has also been found to influence the segregation ratio of markers in typical haploid progeny. This result may also be related to the exclusion or elimination of genetic segments from the persistent diploids. Casual evidence[7] that the *F* polarity of the parents determined the direction of elimination was the prelude to the present report. It should be noted that almost all the crosses cited in previous papers[1–7, 15] have involved $M^- F^+ \times T^- L^- F^-$ parents. The sole exceptions not explicitly labeled involve *filial* $T^- L^- F^+$ stocks which had been remarked[5] as giving distinctive crossing results before their *F* status was learned. It was not possible to study the role of *F* in the determination of hemizygosity in persistent diploids until an empirical method was discovered in this laboratory for the purposeful isolation of F^- "mutants."[10]

Experimental Design, Methods, and Materials.—The cross 1A, M^- *Het* $F^+ \times T^- L^- Th^-$ F^-, was compared with the analogous cross 1B, M^- *Het* $F^- \times T^- L^- Th^- F^+$, which differ solely in *F* polarity. Strict comparability of the parents was insured by deriving the stocks for cross 1B directly from those for 1A: M^- *Het* F^- was secured by the migration method;[10] $T^- L^- Th^- F^+$ was secured by conversion of the corresponding F^- strain by mixed culture in broth with strain K-12. The homogeneous results from separate reisolations of both transformed stocks have been pooled.

The M^- *Het* parent also carried the markers Lac_1^- Mal_1^-; the other parent carried Lac_4^- S^r. The *Lac* markers are very closely linked, permitting balanced diploids of the constitution $Lac_1^- Lac_4^+/Lac_1^+ Lac_4^-$ to be isolated as an appreciable fraction of the occasional lactose-positive prototrophs observed on cross-plates of EMS lactose agar. Media and methods of crossing have been described elsewhere.[5, 11]

Diploid prototrophs were confirmed by their persistent segregation on EMB lactose agar after repeated single-colony purification. These isolates were then tested in replicates on EMB and EMS maltose agar to score their *Mal* character, and by cross-brushing with streptomycin solution, 1 mg/ml, for *S*. Many of the scores were repeated on the diploids after reisolation on EMS lactose agar with unaltered results.

Results and Discussion.—The distribution of *Mal* and *S* among the diploids from the two types of cross is given in Table 1. For the following discussion type "A" will refer to the markers (particularly in the critical region *Mal-S*) that issue from the F^- parent, and type "B" correspondingly for the F^+. These terms correspond loosely to Hayes's usage of "acceptor" and "donor" respectively, terms which suffer, however, from unproved connotations of mechanism. Recombinants displaying one locus from each parent are called "amphitypic."

From Table 1 it will be seen that, with either polarity of F^+/F^-, about 80 per cent of the 618 diploids examined were type A. This result can be equally well explained by prezygotic exclusion or by postzygotic elimination of the type B markers. None of these diploids (or over a thousand previously isolated diploids from similar crosses) were heterozygous for *Mal* or *S*, though for the majority of the

TABLE 1

DIPLOIDS, HEMIZYGOUS FOR *Mal*, *S*, FROM CROSSES OF REVERSED *F* POLARITY

	PARENTS			DIPLOID PROGENY				
	M⁻ Het *Lac₁⁻ Lac₄⁺* *Mal₁⁻ S⁸*		*T⁻ L⁻ Th⁻* *Lac₁⁺ Lac₄⁻* *Mal⁺ Sʳ*	(PROTOTROPHIC *Lac₁⁺* *Mal⁺ Sʳ*	*Mal⁻ S⁸*	*Lac₄⁻/Lac₁⁻* *Mal⁻ Sʳ*	*Lac₄⁺)* *Mal⁺ S⁸*	*Total*
A.	F^+	×	F^-	329 (77.6%) Type A	72 (17.0%) Type B	16 (3.8%) Amphi-I	7 (1.6%) Amphi-II	424*
B.	F^-	×	F^+	16 (8.3%) Type B	164 (84.5%) Type A	8 (4.1%) Amphi-II	6 (3.1%) Amphi-I	194

* In addition, 17 diploids were isolated from a similar cross in which the F^+ parent was inactivated by streptomycin as described by Hayes. Sixteen were type A; one was type B.

isolations these markers were "unselected" in Hayes's sense.[8] But a fifth of the diploids displayed one or more markers from the F^+ parent in hemizygous condition.[12] The type B diploids are evidence that, despite the regular hemizygosity for the *Mal-S* segment, these markers are not regularly excluded from the F^+ gamete.

This conclusion relies upon the preservation of the F^+/F^- polarity of the actual mating events in authentic relationship to the compatibility character of the parent cultures. However, previous experiments[13] have shown that contagion of *F* status does not occur under our conditions of crossing. Table 1 itself excludes contagious reversal of polarity, for over 5 per cent of the diploids were amphitypic, i.e., the elimination had affected one marker from F^+, one from F^-. The type B and amphitypic diploids thus support the hypothesis of postzygotic elimination. To account for the preponderance of type A diploids by an additional exclusion of type B markers from the gametes would be supererogatory on all present evidence.

Table 1 also demonstrates the influence of *F* polarity on the trend of elimination. A possible mechanism for such a conditional control of the polarity of elimination is suggested by the amphitypes, which represent a crossover, prior to elimination, between markers of the two parents. A locus *E* on the type B chromosome may be postulated[14] which breaks after meiosis to result in the loss of the distal segment or

otherwise interferes with normal disjunction. Prior crossing-over between *E* and the markers will result in a type B; crossing-over between markers will give amphitypes; the noncrossovers will of course be type A.

The few remaining markers (out of more than 30 studied) known to be eliminated in diploids, *Hfr*, *Gal*, and Lp_1, are being currently studied by similar methods. *Hfr* is of special interest: as a fixed genetic determinant of compatibility, it might be an allele at the *E* locus itself. However, since elimination occurs in $F^+ \times F^+$ crosses also, in accordance with phenotypic control of compatibility status, it must be assumed that the chromosome on which *E* will break is determined by its transmission from an "effective" F^+, rather than solely by its genetic content. This concept is analogous to the elimination of paternal chromosomes in *Sciara* many cell generations after they have intermingled with their genetically equivalent maternal mates.[9]

The present data display only one aspect of the difficulties inherent in the theory of gametic exclusion. This theory allows for the appearance of type B markers among prototrophic recombinants by suggesting that the relevant chromosomes are only occasionally included in F^+ gametes.[8] To be sure, the likelihood of inclusion is not random, so that a proportion of the gametes must, in fact, be intact, but the probabilities of different chromosome combinations are not fixed by the theory. However, for any pair of linked markers on an "unselected chromosome" the complementary crossover types should occur with equal frequency. This prediction has not been upheld in any well-studied situation, and Rothfels' data on Lac_1 and V_1 show a gross deviation.[15] The theory of prezygotic exclusion of individual chromosomes, furthermore, affords no explanation for the gross deviations from random segregation of heterozygous markers that are an outstanding feature of the diploids[3-5] and are readily explained by the linkage of these markers to haplolethal deficient segments. Nor does it account for the regularity with which some loci are always hemizygous and others never. Our present conclusion on the cytogenetic structure of *E. coli* agrees with a linear sequence in certain segments,[2, 15] but cautions against categorical conclusions of the total chromosome number until the mechanism of elimination has been cleared up. If, as seems likely, chromosome segments rather than intact chromosomes are eliminated, the segregation of any near-by factors will be perturbed even if these are not directly eliminated.

Finally, the mechanical corollary of the pre-exclusion theory, the functioning in *E. coli* K-12 of a virus as a vector of genetic transduction of entire chromosomes, has not been substantiated in fact, by contrast with the simple separation from intact cells of the viral agent of transduction of genetic fragments in *Salmonella*.[16] All the experimental facts of recombination in *E. coli* K-12 are consistent with a mating mechanism whereby an entire nucleus from the F^+ cell is transmitted by a cellular conjugation to the F^- cell. The first evidence of the presumed physiological inequality of the parents was Hayes's demonstration[8] that F^- cells steeped in streptomycin become sexually incompetent, while F^+ cells retain a measure of their vitality.

Summary.—Nondisjunctional diploids from crosses of *E. coli*, strain K-12, are regularly hemizygous for the factors *Mal* and *S*, i.e., are heterozygous for a deletion of this segment. The effect of the sexual polarity of the parents on the polarity of the deletion has been studied. About 80 per cent of the diploid progeny of an

$F^+ \times F^-$ cross retain the segment from the F^- parent. However, 20 per cent retain one or both loci from the F^+ parent. It is therefore concluded that the primary zygote is intact but that the segment from the F^+ parent is preferentially eliminated by a postzygotic process.

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[12] Hemizygosity, as opposed to homozygosity, has been verified by the reversion test only for a sample of Mal^- diploids from both types of crosses, a criterion not directly applicable to Mal^+ isolates or to the linked *S* locus. The extrapolation is justified by the complete absence of *Mal* or *S* heterozygotes and by the application of the reversion test to Mal^- isolates from diploids on which the parents had been reversed in regard to *Mal* (see Lederberg, Lederberg, Zinder, and Lively, *op. cit.*).

[13] For example, $T^- L^- Lac^+ F^-$, $T^- L^- Lac^- F^+$, and $M^- Lac^- F^-$ were grown separately, washed, and mixed for plating according to usual crossing routine. All of several thousand prototrophs ($M^+ T^+ L^+$) were Lac^-, indicating no effective conversion of either of the original F^- cultures. If such mixtures are permitted to grow together for 1–2 hours, an appreciable incidence of conversion is indicated by the appearance of Lac^+ prototrophs. Cf. Lederberg, Cavalli, and Lederberg, *op. cit.*, pp. 723–724.

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